REMARKS/ARGUMENTS

After entry of this paper, claims 1-11, 13-17, 28-37, and 39-44 are pending.

Claim 1 is amended to clarify the language, particularly to clarify the allegedly confusing definition of the term "substitution". The phrase "a substitution which replaces the glutamic acid" is replaced with the phrase -- an amino acid which replaces the deleted glutamic acid...--. This phrase is supported specifically by the examples, as well as the clearly intended meaning of the specification. See, for example, page 4, lines 4-28; page 8, lines 20-24; and the oligonucleotides in Table 1, page 44 of Example 1, which clearly indicate that the codon for Glu was changed to delete the Glu and replace it with His, thereby producing the exemplary mutant E29H.

Similarly the word "mature" is used to modify the "A subunit" to clarify the location of amino acid number 29 of subunit A. The argument for support of this term is discussed in greater detail below. The remaining amendments are minor and inserted to correct the grammar of the claim. Claim 15 is amended to delete subject matter which is redundant with certain subject matter in claim 1.

No new matter is introduced by these amendments.

Allowable Subject Matter

Claims 3, 29, and 44 are objected to as being dependent on a rejected base claim.

The Examiner asserted that the same would be found allowable if rewritten in independent form and to overcome the 35 USC § 112, second paragraph rejection.

Applicants respectfully request reconsideration and withdrawal of this objection for the following reason.

Applicants agree that claims 3, 29, and 44 are novel and non-obvious and therefore allowable. However, since Applicants assert that claim 1 should be found allowable in view of the comments made herein, Applicants would like to defer amending claims 3, 29, and 44 in independent form.

Reconsideration of this objection is requested.

Rejection under 35 USC 112, second paragraph

The Examiner re-instated the previously issued 35 USC § 112, second paragraph rejection as applied to all of the claims.

Claims 1-11, 13-17, 28-37, and 39-44 are rejected under this paragraph. The Examiner asserted that it is unclear as to the meets and bounds of the claimed mutant cholera toxin in view of the following summary and the fact that Subunit A is known to have sequence variations. It is not clear whether the subunit starts with or without the signal sequence. The Examiner indicated that the rejection may be overcome by filing an effective Declaration and inserting a reference sequence into the independent claim.

The lengthy explanation under paragraph 7, pages 3- 7 of the Action by the examiner is summarized as follows:

(1) That Applicants' Declaration incorporating SEQ ID NO: 1 into the specification based upon the incorporation by reference of International Patent Publication No. WO 93/13202(Domenighini) is required to set forth the required statement that the amendatory material being inserted is the material previously incorporated by reference and that the amendment contains no new matter and that the amendatory material consists of the same material incorporated by reference in the referencing application.

(2) *The Examiner asserted that the definition of "substitution" used in the claims includes deletions of amino acids because the WO93/13202 incorporated by reference supports the phrase "at least one additional mutation". By this argument, the Examiner appears to be stating that use by Applicants of the word "substitution" in the claims includes "deletions" prior to the amino acid at position 29, which thereby provides a substitution at that position.*

(3) The Examiner references mutants of CT-CRM produced from two different strains of V. cholera at page 35, a deletion mutant at pages 44-45, and an embodiment at page 108 with different leader sequences. The examiner concludes that Applicants claim a mutant polypeptide of no specified size, but the specification defines mutants as having amino acid substitutions, deletions and additions, defined only by having adjuvanting activity.

Applicants respectfully request reconsideration and withdrawal of this rejection for the following reasons.

In response to the issues summarized in paragraph (1) above, Applicants note that they inadvertently omitted the required statement under 37 C.F.R. §1.57(f) in the Declaration filed in July, 2004, to include essential material from a publication that is not a US patent or US patent publication. Specifically, the Declaration filed by

Applicants on July 12, 2004 inadvertently omitted the statement that (a) the added material was incorporated by reference and that (b) the amendment contains no new matter.

Applicants agree with the Examiner that Applicants incorporated the sequence of SEQ ID NO: 1 from Domenighini. Therefore, Applicants have enclosed a revised Declaration that contains the required information inadvertently omitted from the originally filed Declaration.

Applicants respectfully request that the Examiner consider and enter the enclosed revised Declaration and that the rejection as applied to incorporation of sequence of SEQ ID NO: 1 into Applicants' specification be withdrawn.

In response to the issues summarized in paragraph (2) above, Applicants assert that claims 15 and 16 do not require language that was incorporated by reference from Domenighini in order to be clear. Specifically, the phrase "at least one additional mutation is made to the A subunit of the mutant cholera holotoxin at a position other than wild-type amino acid position 29" in claim 15 is literally supported on page 38, lines 7-10 of the instant specification. Therefore, Applicants would have no reason or need to incorporate the same language from Domenighini.

Applicants direct the Examiner's attention to the fact that amended claim 1 of the present invention specifically notes that "the mutant holotoxin enhances the immune response in a vertebrate host to said antigen". This phrase is literally supported in the specification on page 38, lines 4-6. Therefore, any claims depending therefrom, including claims 15 and 16 which provide a mutant cholera toxin containing an additional mutation, include this requirement. In view thereof, the phrase "enhances the immune response in a vertebrate host to said antigen" was deleted from claim 15. Not only was this phrase deleted in an effort to place the application in condition for allowance, but this phrase is redundant with the similar phrase recited in claim 1.

Further, Applicants have canceled from Claim 1 the term "substitution", which appears to be causing unnecessary confusion in the Examiner's interpretation of the meaning of the claim. The removal of this word, the meaning of which remains contested between Applicants and the Examiner, should moot that ground for rejection. The claim now states that the mutant holotoxin has "an amino acid which

replaces the deleted glutamic acid which naturally occurs at position 29 of the mature A subunit of the wild-type cholera holotoxin". This language should eliminate the controversy regarding whether "substitution" encompasses a deletion mutant only (i.e., one in which the only mutation is a deletion, thus shifting the positions of all subsequent amino acids toward the N terminus) or an insertion mutant (i.e., one which inserts a new amino acid into the sequence without a concurrent deletion, thus shifting the positions of all subsequent amino acids of the sequence toward the C terminus). For example, a deletion or insertion of an amino acid in the sequence preceding wild-type amino acids 1-28 would move the Glu29 to another position, e.g., Glu28 or Glu 30, etc. Thus, this amended language should simplify this examination. This language which requires the deletion of the Glu29 *and* its replacement (i.e., what Applicants mean by a "substitution") is completely consistent with the teachings of the specification, and serves to remove any perceived confusion.

The Examiner has pointed to several sections of Applicants' specification which discuss modifications to a gene, whereby one or more of the non-coding regions is modified, i.e., an existing promoter is substituted for another promoter in the non-coding region. Applicants agree with the Examiner that because the replacement promoter may contain more or less amino acids, the overall number of amino acids changes in the mutant gene. These sections are, however, unrelated to the subject matter of Claim 1.

Further, no section of the specification of the present application, including the Examples, teaches that an amino acid "substitution" of the ***coding sequence***, i.e., subunit A, is a "deletion" only. Specifically, the specification never teaches deleting one of amino acids 1-28, thereby shifting the naturally-occurring amino acid at position 29 of the A subunit to a new position.

As the Examiner is aware, Applicants are free to be their own lexicographers, including using "...terms in a manner contrary to or inconsistent with one or more of their ordinary meanings..." (MPEP 2173.05(a)). Therefore, as discussed in detail above, it is clear from Applicants' specification that the phrase "an amino acid in place of", when utilized to describe ***the claimed subject*** matter, does not include the term "deletion" only, without involving a replacement of the deleted amino acid.

Applicants respectfully request that the Examiner reconsider this point and withdraw the outstanding rejection, as based thereon.

In response to the issues summarized in paragraph (3) above, the claim language now clearly states that the mutant cholera *holotoxin* has "an amino acid which replaces the deleted glutamic acid which naturally occurs at position 29 of the mature A subunit of the wild-type cholera holotoxin". This claim language is believed to be clear and definite for the following reasons. First, the term "holotoxin" as used in Claim 1 inherently defines the claimed biologically active protein as one that is a multimeric protein comprising the assembly of the A and B subunits ***after*** cleavage of their signal peptides, has a 1:5 ratio of A to B polypeptides, is a potent ACP-ribosyltransferase, and binds to one or more species of ganglioside found on eukaryotic cells. Such a definition of the CT holotoxin has been known in the art since before the 1998 priority date of the present application. See, for example, T. D. Connell et al, **1995** Inf. Immun., 63(10): 4091 and 4098 at page 4091, cols. 1 and 2. See, also, M. Sandkvist et al, **1997** J. Bacteriol., 179(22): 6994-7003, see pg. 4997 the sentences spanning the two columns. Applicants have cited these references in the supplemental Information Disclosure Statement attached hereto.

By use of the term "holotoxin" in the claims, the person of ordinary skill in the art clearly would understand that the mutation is present in a full-length, biologically functional cholera holotoxin. Since the term "holotoxin" has an accepted meaning in the art, one of skill in the art would readily be able to practice the claimed invention without inserting the sequence of one specific wild-type holotoxin into the claim or without providing the size of the claimed mutant CTs of Applicants' invention. While all mature CT subunit A polypeptides are not identical in amino acid length, as argued previously, all known CT subunit A polypeptides to Applicants' knowledge have a signal sequence and have as the 29th amino acid of the mature polypeptide (i.e., after removal of the signal peptide), a glutamic acid residue. This can be readily determined by a simple alignment of the sequences. See, for example, the result of the alignment of the sequence of SEQ ID NO: 1 from Domenighini with the Swiss Protein Accession Nos. P01555, Q8VL16 and Q8L356, referenced by the examiner. Note that SEQ ID NO: 1 is also the subunit A sequence of Mekalanos et al, **1983** Nature, 306:551-557 minus the 18 amino acid signal sequence, and starting at the Asn

residue under which Mekalanos inserts a "1", indicative of the first amino acid of the mature A subunit. Since SEQ ID NO: 1 clearly begins with the first mature Asn residue and that residue is the same residue identified by Mekalanos, the use of the term "mature" preceding "A subunit" in Claim 1 is not new matter.

Specifically, using knowledge of those of ordinary skill in the art, known published CT A subunit variants, and the teachings of Applicants' specification, one would readily be able to locate the naturally occurring Glu at position 29 in the A subunit of any of the wild-type cholera holotoxin variants. Such a task is well within the skill of one of ordinary skill in the art.

The Examiner noted that the only "reference point" for the claimed mutant is that of the wild-type cholera toxin. Applicants would like to again emphasize that the claimed subject matter is drawn with reference to wild-type cholera holotoxin subunit A. Further, the sequence of a wild-type subunit A has been known since at least the 1983 publication of Mekalanos, recited as publication 1 in the bibliography of the specification, as well as the 1993 publication date of Domenighini. It is well known in the art that all of the known variants of cholera holotoxin subunit A have a Glu at their naturally-occurring (or wild-type) residue position 29. In fact, the same convention for identifying amino acid positions of cholera toxin subunit A is used for all CT subunit A sequences known in the art. Therefore, one of skill in the art would readily be able to locate the Glu at wildtype position 29 in the mature A subunit of any cholera toxin CT and substitute the same with an amino acid other than aspartic acid.

Further, the specification clearly teaches how to evaluate whether the mutant CT-CRMs are biologically active by using the illustrated ganglioside Gm1 binding assay and the Y-1 adrenal tumor cell assay, described in the specification. In view thereof, given the teachings in the specification and knowledge in the art, as identified above, one would be able to practice the claimed invention to obtain a mutant CT-CRM meeting both the structural and functional requirements of the claims without unnecessarily (a) inserting the sequence of one specific wild-type holotoxin, such as that of SEQ ID NO: 1, or (b) restricting the size of the claimed mutant CTs of Applicants' invention.

Applicants therefore respectfully request that this rejection be reconsidered and withdrawn.

Specification Objection

The Amendment filed July 12, 2005 is objected under 35 USC § 132(a).

The Examiner asserted that SEQ ID NO: 1 is not supported by the original disclosure and is new matter. The Examiner further asserted that the same was improperly incorporated by reference.

Applicants respectfully request reconsideration and withdrawal of this rejection for the following reason.

As noted above, Applicants have enclosed a revised Declaration that properly incorporates by reference the sequence provided in the sequence listing filed on July 12, 2004 and identified as SEQ ID NO: 1.

Reconsideration of this rejection is requested.

35 USC § 102(b) Rejections

Claims 1-2, 4, 6-8, 11, 13-17, 28, 30, 32-35, 37, and 39-43 are rejected under 35 USC § 102(b) over International Patent Publication No. WO 95/17211 (Rappuoli) as evidenced by the sequence for cholera toxin and E. coli heat labile enterotoxin of Zhang et al for reasons of record.

The Examiner further asserted that the definition of "substitution" in Rappuoli on page 7, lines 5-24 includes deletions of amino acids.

Applicants respectfully request reconsideration and withdrawal of this rejection for the following reason.

Rappuoli does not anticipate the present invention. Rappuoli teaches an *E. coli* labile toxin LT mutant having a Glu-Lys mutation at position 112, and an Arg to Lys substitution at position 7; and a pertussis toxin mutated with a Glu to Gly mutation at position 129 or an LT with an Arg-Lys substitution at position 192. Nowhere in Rappuoli is there any reference to a cholera toxin subunit A with a mutation in which another amino acid is in place of the naturally occurring Glu at position 29, which is a requirement of Applicants' mutant cholera holotoxin. In fact, Rappuoli says absolutely nothing about the wild-type residue at position 29 of the CT

subunit A and thus cannot teach a mutation at that point. Rappuoli does not teach that the Glu at wild-type position 29 is no longer present in the claimed mutant cholera holotoxin sequence.

Therefore, Rappuoli alone or taken with Zhang does not anticipate the claims of the present invention.

Reconsideration of this rejection is requested.

Claims 1, 2, and 13 are rejected under 35 USC 102(b) over Glineur for reasons of record.

The Examiner further asserted that Glineur produced mutant cholera holotoxin with the amino acid at position 29 replaced through deletion of an amino acid, which resulted in a tyrosine existing at position 29.

Applicants respectfully request reconsideration and withdrawal of this rejection for the following reason.

As previously noted, Glineur does not teach an antigenic composition which contains a first antigen and a mutant cholera holotoxin (CTX) that has an "adjuvant" effect on the first antigen. Glineur even distinguishes the differences between a substitution, i.e., an amino acid in place of the Glu, and deletion by the preparation of the E29Δ **deletion** mutant and the E29D substitution mutant.

Therefore, in view of Applicants' amended claims and the arguments addressed above, for the reasons stated above with respect to Rappuoli, and the reasons provided above, Glineur does not teach Applicants' ***claimed*** invention.

Reconsideration and withdrawal of this rejection is respectfully requested.

The Director is hereby authorized to charge any deficiency in any fees due with the filing of this paper or during the pendency of this application, or credit any overpayment in any fees to our Deposit Account Number 08-3040.

Respectfully submitted,

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Attachments:

(1) Declaration
(2) Supplemental Information Disclosure Statement and references